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THE OCCURRENCE OF OVULATION IN THE RABBIT AS A RESULT OF STIMULATION OF THE CENTRAL NERVOUS SYSTEM BY DRUGS

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Three years ago Marshall & Verney [1936] reported the results of experiments in which the effects of electrical stimulation of the central nervous system on the occurrence of ovulation in the rabbit were investigated. It was shown that ovulation and pseudo-pregnancy could readily be induced by these means, and favour was given to the view that the electrical stimulus produced these sexual changes by the functional intermediation of the anterior pituitary body. An attempt to produce ovulation in the rabbit on heat by the action of certain drugs which might conceivably, by direct or indirect means, bring about an increased activity of the anterior pituitary, proved unsuccessful, the drugs chosen being pilocarpine, eserine, acetylcholine and adrenaline.

It seemed to us of interest to see whether ovulation in the rabbit on heat could be induced by drugs with convulsive or other stimulating action on the central nervous system, thereby initiating by chemical means a train of events similar to that produced by an electrical stimulus. With this object in view we have used the following drugs: strychnine, apomorphine, β-tetrahydronaphthylamine, ergometrine, carbaminoylcholine hydrochloride, coriamyrtin and picrotoxin.

Method

The does were isolated for at least four weeks before use, and in order to prevent the occurrence of single haemorrhagic follicles, frequently found in such rabbits, more than half the animals were copulated with a vasectomized buck about 22 days before experiment. Post-mortem

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examination of the ovaries was carried out usually 48, and sometimes 24 or 72 hr. after the injections. For the microscopical examination the ovaries were fixed in Bouin's fluid and stained with haematoxylin and eosin. All injections were made into the ear vein.

Results

(*a*) *Picrotoxin.* The dose of picrotoxin was 0·9–1·1 mg./kg. body weight: this was lethal in about 20 % of the cases and convulsive in about 75 %. An attempt to prevent death by giving a small dose of ether whenever the convulsions began to involve the respiratory muscles, was made in all instances, but was not always successful.

Table I summarizes our results on the rabbits injected with picrotoxin only. The rabbits showing convulsions are treated separately from those in which salivation, strange posture and excitement were the only signs

Table I

	Convulsions present				Convulsions absent			
Ovulation observed	Follicles enlarged	Follicles haemorrhagic	Death from injection	No reaction of ovaries	Ovulation observed	Follicles enlarged	Follicles haemorrhagic	No reaction of ovaries
135 (ovulation imminent)	158	141	136	143	151	148	142	153
139	—	145	150	183	—	206	144	209
140	—	146	152	195	—	207	210	—
155	—	184	154	196	—	216	215	—
211	—	203	176	197	—	—	—	—
217	—	—	200	204	—	—	—	—
—	—	—	212	205	—	—	—	—
—	—	—	213	208	—	—	—	—
—	—	—	—	214	—	—	—	—
6	1	5	8	9	1	4	4	2

In all animals included in this table the dose of picrotoxin was 0·9–1·1 mg./kg. With the exception of the rabbit in which the reaction is described above as "ovulation imminent" the ovaries were examined 48 or 72 hr. after injection. The index number of each rabbit is given in the columns above, and in the last row are the numbers of animals which showed the effects described at the head of each column.

exhibited. If one discards those dying from the injections, there remain twenty-one rabbits which survived convulsions: six of these ovulated, and six showed batches of enlarged, cystic or haemorrhagic follicles, reactions which are usually considered as proof of subliminal stimulation of the ovary by the anterior lobe hormone. The nine rabbits which showed no signs of ovarian reactions were found to be either pseudo-pregnant, or not on heat as judged from the colour of the uterus and vulva. Whereas in this group of twenty-one animals six ovulated, one only did so in the

other group (eleven animals) comprising those rabbits which did not show convulsions. With the exception of two rabbits, however, which were not on heat, all the others showed follicle growth or haemorrhage: the occurrence of convulsions is not, therefore, a condition necessary for the appearance of an ovarian reaction.

In those rabbits in which ovulation occurred, between three and five ovulating follicles were usually present in each ovary: in this respect the picture was the same as in normally induced ovulation. Occasional haemorrhages, however, into single follicles indicated that these ovulations were not entirely normal. Moreover, the time course of the pharmacologically produced ovulation was always abnormal: if the rabbits (see no. 135, Table I) were killed 24 hr. after injection, ovulation was only imminent, and not before 48 hr. after treatment with the drug was the process certainly completed. Compared with the natural latency of 10 hr. between copulation and ovulation, the period between chemical stimulus and ovarian reaction is very much longer, longer even than that found by Marshall & Verney [1936] in their experiments on the production of ovulation by electrical stimulation of the central nervous system.

(*b*) *Other substances.* A series of other drugs was tried in order to discover whether any substance other than picrotoxin would produce

TABLE II. No ovarian response was seen to these drugs when given in the dosage shown

No. of rabbit	Kind and dose of drug	mg./kg.	General effects
133	Strychnine HCl	0·15	Excitability
134	Do.	0·17	Convulsions
147	Apomorphine HCl	10·0	Violent excitement
149	Do.	10·0	Do.
156	β-Tetrahydronaphthylamine	12·0	Excitement and rise in temperature
159	Ergometrine	2·0	Exophthalmos, dilatation of the pupils, increase in respiratory rate and in temperature
160	Do.	2·0	Do.
163	"Doryl" (carbaminoylcholine HCl)	0·02	Salivation, defaecation, vasodilatation of ear vessels
165	"Doryl" (after 5·0 mg. atropine sulphate) followed by "Doryl" (after 6·0 mg. atropine sulphate)	0·05 0·05	— —
168	Picrotoxin (after 20 mg. atropine sulphate)	0·8	Convulsions
169	Do.	0·8	Do.
170	Do.	0·8	Do.
171	Do.	0·8	Do.
172	Coriamyrtin	0·25	Do.
174	Do.	0·25	Do.

ovulation in does on heat. The results are summarized in Table 2: as will be seen, they were entirely negative. Adrenaline and a number of stimulants of cholinergically innervated effector organs had previously

been found ineffective by Marshall & Verney [1936]. "Doryl" (carbaminoylcholine HCl), tested on rabbits 163 and 165, is another instance of an ineffective parasympatheticomimetic drug. The larger doses of this drug were preceded by injections of atropine, since they otherwise would have proved lethal. This procedure, however, is open to the criticism that the atropine may have interfered with the ovulation: indeed, Foster, Haney & Hisaw [1934] report that after injections of atropine the mating of does fails to produce ovulation. In our experience, however, ovulation may occur even when doses of atropine as high as 20 mg. are given about 10 min. before the mating, but difficulties arise from the fact that such treatment is frequently followed by a refusal to mate.

An attempt to test the effect of atropine on ovulation produced by picrotoxin gave inconclusive results, since the convulsive and lethal effect of the drug was increased by the atropine. It was necessary, therefore, to give less picrotoxin, i.e. 0·8 mg./kg. in the presence of atropine: in spite of violent convulsions, none of the rabbits so treated ovulated. Since this dose of picrotoxin does not produce ovulation in the absence of atropine, its failure to do so in the presence of atropine may be the simple result of the smaller dose of picrotoxin. These results may, however, be regarded as a further indication that no correlation exists between the convulsive action of picrotoxin and its effect on the ovaries.

Another example which shows that ovulation is not a simple consequence of any kind of convulsions is given by the result of similarly conducted experiments with strychnine (rabbits 133 and 134) and with coriamyrtin. The negative results with the latter drug are somewhat striking, since both picrotoxin and coriamyrtin appear to have similar actions on the central nervous system (see Swanson & Chen [1936]). Moreover, the effects both of apomorphine in a dose which produced violent general excitement (rabbits 147 and 149), and of two drugs which act on the sympathetic centres (β-tetrahydronaphthylamine (rabbit 156), and ergometrine (rabbits 159 and 160)), proved equally negative in this regard.

Discussion

Picrotoxin, given intravenously to rabbits on heat, may produce follicle growth, follicle haemorrhages or ovulation. The absence of response to injections of a series of other drugs with a stimulating action on the central nervous system may be of significance: no definite conclusions from such negative experiments can, however, be drawn, since the experiments with picrotoxin have shown that positive results are only obtained within a very limited range of dosage and when the does are

fully on heat. Though most of the drugs were given in the highest amounts compatible with survival, the absence of response might be due to failure in finding the optimal conditions for their action. The most reasonable interpretation of our results, however, would appear to lie in a more elective action on the innervation of the anterior lobe of the pituitary by picrotoxin than by the other drugs whose action in this regard we have investigated.

SUMMARY

Picrotoxin in a dose of 0·9–1·1 mg./kg. was injected intravenously into rabbits on heat. Follicle growth, development of batches of cystic and of haemorrhagic follicles, or ovulation ensued in a large number of animals so treated. No ovarian responses were obtained to the injection of a series of other substances, most of which were stimulants of the central nervous system.

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